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Cord-forming Palaeozoic fungi in terrestrial assemblages

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Summary: The fossil record paints a thin picture of early terrestrial life. Useful diagnostic features are rare in the organic-walled fossils of the first land colonizers, and at first glance the Silurian–Devonian *Tortotubus protuberans* seems no exception. Now, new material from New York, Gotland and Scotland reveals the ontogenesis and affinity of this problematic organism. Its filamentous early stages (previously referred to *Ornatifilum lornensis*) demonstrate simple septal perforations and a bi-layered cell wall; threads of entwined filaments, bounded by an elaborately sculptured surface, arose through the retrograde growth and subsequent proliferation of secondary branches. Taken together, this morphology and pattern of growth indicates an affinity with the 'higher' fungi (Dikarya) and documents the formation of differentiated mycelial tissue. The presence of complex mycelial fossils in the earliest Silurian corroborates the likely contribution of fungi to the colonization of land and the establishment of modern sedimentological systems; their rise seemingly accompanied the diversification of early embryophytes and the vegetation of the terrestrial biosphere.

Keywords: Fungal evolution, land colonization, Dikarya ('Higher Fungi'), Ascomycota, Basidiomycota, mycelial cords

Introduction

The fossil record of life's earliest steps onto land is profoundly incomplete. Plant macrofossils occur from the mid-Silurian (Edwards, Feehan, & Smith, 1983), although dispersed spores document probable land plants from the Middle Ordovician (Wellman & Gray, 2000). Whereas fungi were undoubtedly a component of these early terrestrial ecosystems, they left no verifiable fossils until the Early Devonian (Taylor *et al.*, 2003) – by when all major groups except the Basidiomycota are represented. Silicified and charcoalified lagerstätten record a range of fungal ecologies, including mycorrhization, lichenization, saprophytism and possible fungal rhizomorphs (Taylor *et al.*, 2003; Edwards & Axe, 2012; Edwards, Axe, & Honegger, 2013); there is even equivocal evidence of macroscopic fungal bodies (*Prototaxites*) (Boyce *et al.*, 2007; Edwards & Axe, 2012).

This abundant diversity hints at an unseen past – an insinuation that is echoed by molecular clocks. The split between the terrestrial phyla Basidiomycota and Ascomycota is dated to between 450–2000 Ma (mid-Ordovician–Palaeoproterozoic), the wide range reflecting sensitivity to calibration (Taylor & Berbee, 2006); the younger estimates seem more robust (Lucking *et al.*, 2009; Beimforde *et al.*, 2014) but still imply a long and unfossilized pre-Devonian history. Admittedly, long ghost lineages are the norm among Fungi: Basidiomycota must have diverged prior to the Ascomycota radiation recorded in the Early Devonian Rhynie Chert (Taylor *et al.*, 2003), even though they left no unambiguous fossils until the Carboniferous (Dennis, 1970; Krings *et al.*, 2011). In part, this reflects obstacles to preservation. A graver difficulty, however, is the shortage of morphological characters that are both prone to fossilization and diagnostic of a single group. Prior to the Devonian, such characters are particularly scarce, and not one of the putative candidates (Retallack, 1994; Redecker, Kodner, & Graham, 2000; Peterson, Waggoner, & Hagadorn, 2003; Butterfield, 2005; Ivarsson *et al.*, 2013) can be confidently assigned to the Fungi (Butterfield, 2005, 2015; Antcliffe & Hancy, 2013).

The most promising pre-Devonian fungi are septate filaments from near-shore Silurian deposits referred to *Ornatifilum lornensis* (Wellman, 1995), which bear conidia-like ampullate branches and perforate septa – but these characters are also present in certain red algae, so a fungal identity cannot be considered secure. The present study examines new Silurian–Devonian material from the Burgsvik Beds, Gotland, Sweden; the Lower Old Red Sandstone, Kerrera,

Scotland; and the Catskill Delta succession, Albany, eastern New York State, USA. New specimens demonstrate that *O. lornensis* develops into the problematic microfossil *Tortotubus protuberans*, previously reported from the Ordovician and Silurian (Johnson, 1985; Thusu *et al.*, 2013). The organism exhibits a distinctive growth trajectory involving elaborate branching and the formation of multi-filament strands. Together, these features suggest that *Ornatifilum* (=*Tortotubus*) may be an early terrestrial fungus.

Materials and methods

The green, poorly consolidated mudstones of the Mid-Ludfordian (Late Silurian) Burgsvik Formation (Husryggen, Gotland, Sweden: 56°56'35" N, 18°09'00" E) belong to a sequence of sandy and muddy strata deposited in a marginal marine setting that periodically encountered terrestrial exposure (Calner, Jeppsson, & Munnecke, 2004). Laminated green-grey siltstone horizons from the Lower Old Red Sandstone at Port Dubh, Kerrera, Argyll, Scotland (56°22'52" N, 05°34'38" W) date to the earliest Devonian or latest Silurian, and were deposited in an alluvial setting, in ephemeral freshwater lakes with no marine influence (Trewin *et al.*, 2012). Green mudstones from Bates Hollow Quarry, Albany, Eastern New York (42°26'11" N, 74°15'03" W) were deposited in a Givetian (upper Mid Devonian) delta system, and represent overbank deposits, estuaries or short-lived lakes (VanAller Hernick, Landing, & Bartowski, 2008). Whereas the Burgsvik Formation contains organic-walled microfossils of marine and terrestrial organisms (Calner *et al.*, 2004; Hagström & Mehlqvist, 2012; Smith & Butterfield, 2013), the Kerrera biota and the Albany microfossil flora are exclusively non-marine (VanAller Hernick *et al.*, 2008; Trewin *et al.*, 2012). The fossil material is thus reasonably interpreted as a terrestrial organism that was occasionally washed in to estuarine or marine settings.

Mudstone chips were dissolved in concentrated (40%) hydrofluoric acid and the resulting slurry washed on a 62 µm sieve. Following transfer to distilled water, organic-walled microfossils were hand-picked by pipette and mounted on glass slides. Around 250 specimens were permanently set for light microscopy using heat-setting resin, and *c*. 50 were prepared for SEM analysis. Specimens are deposited at the Swedish Museum of Natural History (SMNH) and the Sedgwick Museum, Cambridge (CAMSM).

The possibility of contamination can be discounted based on the presence of diagenetic pyrite grains, the thermal alteration index of the material (Burgess, 1974) (which corresponds to that of co-occurring kerogenous microfossils; see Supporting Information Figs S1–S3), and the independent recovery of equivalent material from the same strata (Sherwood-Pike & Gray, 1985; Wellman, 1995).

Results

Systematic Palaeontology

Kingdom Fungi Moore 1980 (Moore, 1980)

Total-group Dikarya Hibbett *et al.* 2007 (Hibbett *et al.*, 2007)

Tortotubus Johnson, 1985

Tortotubus protuberans Johnson, 1985

1969 Filaments – (Lele & Streel, 1969) fig. 76

1978 Hyphal filaments with septa – (Pratt, Phillips, & Dennison, 1978) pl. 4.4–4.8

1985 *Tortotubus protuberans* Johnson 1985 – (Johnson, 1985) pls. 11.5, 14

1985 Narrow septate tubule – (Johnson, 1985) pl. 13.3

1985 Hyphae – (Sherwood-Pike & Gray, 1985) Figs 2a–f, 3, 4

1990 *Tortotubus protuberans* – (Richardson & Rasul, 1990)

1995 *Ornatifilum lornensis* Wellman 1995 – (Wellman, 1995) pl. 5

1997 *Ornatifilum lornensis* Wellman 1995 – (Hagström, 1997) fig. 8r–s

2007 *Tortotubus protuberans* Johnson 1985 – (Steemans, Wellman, & Filatoff, 2007)

2013 *Tortotubus protuberans* Johnson 1985, *Tortotubus protuberance [sic]* – (Thusu *et al.*, 2013)

Revised diagnosis.—Branched filaments, 5–12 µm in diameter, that are variably smooth-walled or covered with an irregularly distributed external ornament that may comprise grana, coni, spinae, verrucae and occasionally pila. Lateral branches occur immediately prior to simple perforated septa at high angle to main filament, from which they are separated by a basal septum; their first cell is often flask-shaped. Secondary branches arise from the proximal cell of secondary branches and grow along main filament, commonly in retrograde direction. Secondary branches amalgamate to form a roughly tubular envelope up to 40 µm wide, the surface of which is convoluted or sinuous with hollow papillae that are 1 to 6 µm high and from 1.5 to 3 µm wide.

Occurrence.—Tortotubus has only been reported from marginal marine (Sherwood-Pike & Gray, 1985; Hagström, 1997; Calner *et al.*, 2004; Le Hérissé, Paris, & Steemans, 2013; Thusu *et al.*, 2013) and terrestrial (fluvial or lacustrine) settings (Pratt *et al.*, 1978; Richardson & Rasul, 1990; Wellman, 1995; Trewin *et al.*, 2012) and is consistently associated with terrestrially-derived palynomorphs (Pratt *et al.*, 1978; Johnson, 1985; Richardson & Rasul, 1990; Wellman, 1995; Hagström, 1997; Steemans *et al.*, 2007; Le Hérissé *et al.*, 2013). It exhibits a broad geographic and temporal occurrence, spanning the recently formed Laurussian supercontinent (McKerrow *et al.*, 2000) from the onset of the earliest fossilized plant communities in the Llandovery (Early Silurian) to the rise of the earliest forests in the Givetian (Middle Devonian). Additional material from Chad and Saudi Arabia (Le Hérissé *et al.*, 2013; Thusu *et al.*, 2013) is difficult to date precisely, but conceivably extends the stratigraphic range of *Tortotubus* into the latest Ordovician.

Description.—The fossils comprise elongate filaments with a uniform width of 5–12 µm. The external surface is typically ornamented with an irregular array of globose bodies (Figs 1–3, S1–S4), but the internal surface is smooth (Fig. 1a). The density of ornament varies; in places it is absent entirely (Figs 2b; S1b–d, S2e, S3o). The filament wall comprises two layers; the boundary of the layers bears a subtle ornament of pits with raised rims (Fig. 1d). Perforate septa occur every 150 µm (Figs S1a, S2a–b, p, S3a–d); the filament shows no variation in thickness near septa. Each septum is perpendicular to the filament wall and is flat, with no rim around the central pore – which occupies approximately the central third of the septum (Fig. 1b). Septa are single; the 'double septum' of Sherwood-Pike & Gray (1985) reflects an artefact of oblique compaction.

One to three branches arise from the main filament immediately prior to each septum (Figs 2a–d, 4b–d; S1b–d, S2e, S3o). Branches are angled at 45–90° from the direction of the main filament; in the third dimension they are separated by about 120° around the main filament. These primary branches rapidly increase in width before gradually tapering to the same width as the parent filament (Figs 1c; 2a–d; 3; S1d–e, S2, S3b, g, k, m, S4–S5). A septum often marks the point at which this narrower width is reached, typically *c.* 20 µm from the parent filament (Figs 2a, d; S1d, S5e). The primary branches are commonly truncated shortly after this septum, leaving an irregular edge suggestive of breakage; this produces the ampulliform shape described in Sherwood-Pike & Gray (1985) (Fig. 4a–d). Unbroken filaments, however, terminate in a

semicircular tip identical to the termini of primary and tertiary branches (Fig. 2d; S1d, S2e, S4b). No ascospore-like structures were observed (cf. Sherwood-Pike & Gray, 1985), but three specimens did preserve a 25 µm-wide laevigate sphere at the end of a filament (Figs 2g, 1e).

The proximal region of a primary branch often gives rise to one or two secondary branches (Figs 2a–d, 3a–d, 4a–e; S1b–d, S3a, b, d–e, g, i–p, S4b–c). These typically exhibit retrograde growth alongside the nearest filament. They are lighter in colour and more transparent than other filaments, and deform more strongly about included pyrite crystals – indicating a less robust wall construction. Their globular surface ornament is sparser or altogether absent (Figs 2b–f, 3c; S1b–d, S3o, S4b–c). Secondary branches adhere to the main filament and to each other by means of pustular growths (Figs 2d, 3c, 4c).

Secondary branches are themselves overgrown by further secondary branches originating more distally along the main filament (Figs 4d; S3b, j, S4b–c). As secondary branches accumulate, they interact with one another; their surfaces amalgamate to form an envelope, up to 40 µm wide, that envelops the main filament and its primary branches (Figs 2e–j, 3e–j, 4f–g; S 2c–d, S3d, h–n, q, S4b–c, S5). This encompassing envelope develops an superficial ornament of narrow, intertwining, branching filaments (Figs 2g, i–j; 3f–i, 4f–h; S3h, q, S4c, S5c–d) and pronounced (c. 1 µm) hemispherical papillae (Figs 2h, 3j, 4g; S3l, S5c–d).

Reconstruction.—The morphological series evident in *Tortotubus* documents the formation of cords by the accretion of secondary branches around a single leading filament (Fig. 4a–h). The distal components of the organism presumably comprised single filaments with regularly spaced primary branches (Fig. 4a); as the terminal growth zone advanced, secondary branches arose from the basal regions of the primary branches and grew in the opposite direction to the original filament (Fig. 4b). As these secondary branches accumulated (Fig. 4b–e), filaments amalgamated into a single fused cord with a surface texture comprising pustules and anastomosing tendrils (Fig. 4f–h).

Discussion

Affinity.—The only groups known to exhibit punctate septa are the red algae and ‘higher’ fungi, and *Tortotubus* can reasonably be assumed to belong to one of these two taxa. The case for a red

algal affinity is underwhelming: rhodophytes exhibit neither differentiated filaments nor complex surface ornament; their pit connections are an order of magnitude smaller than the septal pores of *Tortotubus*; and their distally bifurcating branching pattern is difficult to reconcile with the lateral branching and retrograde growth observed in the fossil material. In contrast, the higher fungi (Dikarya, =Neomycota, comprising Basidiomycota + Ascomycota) provide a compelling analogue: a polyphyletic assemblage of taxa produces lateral branches just prior to septa; a globose ornament is often present (Fig. 4i–m, refs. (Ainsworth, Sparrow, & Sussman, 1973; Fox, 1987)); hyphae grow apically; the fungal cell wall comprises compositionally distinct layers (Ruiz-Herrera, 2012); the width of fungal hyphae is uniform about septa; and septa are usually perpendicular to cell walls and have a large central aperture (Ainsworth *et al.*, 1973; Cavalier-Smith, 1998). (This does not necessarily indicate a close relationship to any individual taxon – merely that the *Tortotubus* growth regime falls within the paradigm of fungal behaviour.)

The absence of unambiguous reproductive structures makes further taxonomic resolution difficult to achieve. Whereas the primary branches were previously identified as conidia (Sherwood-Pike & Gray, 1985), conidia do not give rise to secondary branches. The ampulliform shape of primary branches instead results from breakage: the tip of each branch was originally a rounded filament tip (Fig. 2d) rather than a spore-bearing structure. More generally, the absence of clamp connections and dolipore septa seems to rule out certain positions within Basidiomycota, but these features are frequently unexpressed or secondarily lost (Bauer, Oberwinkler, & Vánky, 1997); their absence by no means excludes *Tortotubus* from the Basidiomycota crown. On the available information, then, *Tortotubus* may be aligned with Ascomycota or Basidiomycota, or may occupy the Dikarya stem group.

Pattern of growth.—The pattern of growth in *Tortotubus* finds a vivid analogue among mushroom-forming members of Basidiomycota and Ascomycota that produce coherent threads of entwined hyphae – termed mycelial cords, mycelial strands, or syrrotia. The leading edge of a mycelial colony consists of individual hyphae; some distance behind the leading edge, ‘following’ (‘tendril’ or ‘runner’) hyphae, corresponding to the secondary branches in *Tortotubus*, branch off and grow alongside an ‘adopted’ leading hypha (Butler, 1966). ‘Following’ hyphae, which represent the only documented occurrence of sustained retrograde growth among fungi (Haskins, 1967), are typically narrower than the adopted hypha; they may fuse, anastomose or branch. The form of *Serpula lacrymans* (Basidiomycota) directly parallels

that of *Tortotubus* (Fig. 4a–l): primary branches emerge in whorls immediately behind septa in the main hypha; secondary branches emanate from the base of the primary branch and follow the main hypha, anastomosing to form a mantle (Butler, 1958). *Phymatotrichopsis omnivora* (Ascomycota) produces cylindrical cords with a differentiated surface formed of anastomosing ancillary hyphae (Rogers & Watkins, 1938; Butler, 1966; Alderman & Stowell, 1986), akin to the distinctive surface of mature *Tortotubus* cords. This similarity does not necessarily indicate a close relationship, but does show that Dikarya can produce the range of morphologies expressed by *Tortotubus*.

Tortotubus fossils are thus reconstructed as components of a fungus-grade mycelial network. Mycelial cords are often associated with a terrestrial habit (Watkinson, 1999), where they allow subaerial fungi to colonize contiguous surfaces. Reconstructing such a habitat for *Tortotubus* accounts for its consistent occurrence alongside unambiguously terrestrial plant fossils (Pratt *et al.*, 1978; e.g. Wellman, 1995; VanAller Hernick *et al.*, 2008), and for its occurrence in the exclusively non-marine Lower Old Red Sandstone (Trewin *et al.*, 2012) alongside the periodically-exposed marginal-marine Burgsvik beds (Calner *et al.*, 2004). Cords are typically associated with saprotrophic decomposition of plant material; they allow a colony to relocate nutrients from depleted to pristine resources (Boddy, 1993), and thus represent a specialization for environments where nutrients are scarce and heterogeneously distributed. As such, *Tortotubus* may reasonably be interpreted as a terrestrial, possibly saprotrophic, 'higher' fungus.

Implications.—*Tortotubus* fills an important gap in fungal evolution: it ranks among the earliest body fossils of non-marine eukaryotes, and may represent the oldest fossil fungus. The identification of mycelial networks is particularly significant at this formative stage of the subaerial biogeosphere. Mycelial cords serve as a buffer for nutrient levels in the immediate subsurface, storing and transporting nutrients – whether by transport through a mycelial network, or through consumption by invertebrates (Boddy, 1993). *Tortotubus* (like modern fungi) conceivably contributed to nutrient cycling, encouraging the growth and diversification of plant communities (Cheplick, 2004; Sanders, 2004); it may also have been a nutrient source for pioneering land animals (MacNaughton *et al.*, 2002). In contrast to early plants, which lacked roots and thus had limited interactions with the subsurface (Raven & Edwards, 2001), mycelial fungi play an important role in stabilizing sediment (Neuman & Maxwell, 1999), encouraging

weathering (Verrecchia, 2000), and forming soils (Gupta & Germida, 1988). As a widespread and enduring component of the rapidly diversifying Silurian–Devonian subaerial ecosystems, *Tortotubus* emphasizes the potential role of fungus-grade organisms in the mid-Palaeozoic shift in global sediment characteristics and the coeval rise of complex terrestrial ecosystems.

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Figure Legends

Figure 1. *Tortotubus* from the Burgsvik Formation, Gotland (SEM). (*a*) SMNH S038211-01. Globose ornament on outer cell wall; inner cell wall is smooth. (*b*) SMNH S142804-03.

Perforate septum exposed by detachment of primary branch. (*c*) SMNH S038212-01. Aperture in primary branch, interpreted as attachment point of secondary branch. (*d*) SMNH S038211-02. Inner and outer layers of cell wall; arrowheads indicate pits with raised rims on inner wall layer. Bars, 3 μm.

Figure 2. Development of cords in *Tortotubus protuberans* from the Burgsvik Formation, Gotland (transmitted light microscopy with focal stacking). Presumed growth direction to top of page. (*a*) SMNH S038213-01. Simple filament, with nascent secondary branches near base of primary branches. (*b–c*) SMNH S038214-01: *(b)* Secondary branch growing towards main filament; (*c*) secondary branch commencing retrograde growth alongside main filament. (*d*) SMNH S038215-01. Pustules (arrowheads) connecting secondary branches to main filament. Left-hand primary branch has intact tip. Tip of secondary branch approaching node from above. (*e*) SMNH S038216-01, S038217-01. Secondary branches forming envelope about main filament. (*g*) SMNH S038218-01. Filament with envelope of anastomosing branches. Spheroid structure at end of primary branch. (*h*) SMNH S038219-01. Regular pustular ornament on envelope surface. (*i*) SMNH S038220-01. Wide filament; a further envelope overlies anastomosing filaments and pustules. (*j*) SMNH S038221-01. Envelope of secondary filaments covering main filament and primary branches, ornamented by narrow anastomosing filaments. Abbreviations: 1°, primary branch; 2°, secondary branch; ana, anastomosing branches; env, envelope; mf, main filament; pust, pustule; pyr, pyrite cuboids; sep, septum. Bar, 50 μm.

Figure 3. Cord formation in *Tortotubus* from the Burgsvik Formation, Gotland (SEM). (*a, b*) SMNH S038222-01; secondary branches propagate from basal region of primary branch and proceed down stem to next branching point. (*c*) SMNH S038211-03; surface of secondary branch fuses with surface of main filament. (*d*) SMNH S038223-01; extended filament showing extent of secondary branch growth. (*e*) SMNH S038224-01; secondary branches beginning to form envelope with ornamented surface; primary branches not incorporated. (*f*) SMNH S03821-02; envelope of secondary branches encloses primary branches. (*g*) SMNH S038223-02; fine anastomosing filaments on surface of envelope. (*h*) SMNH S038223-03; anastomosing filaments fusing to produce distinctive surface ornament. (*i*) SMNH S038223-04; development of surface ornament from anastomosing

filaments. (*j*) SMNH S038225-01; surface ornament of intertwined anastomosing filaments and subregular pustules. Abbreviations as in Fig. 2. Bars, 20 µm.

Figure 4. Growth in *Tortotubus* and analogous morphology in modern cord-forming fungi. (*a–h*) Schematic illustration of cord formation in *Tortotubus*. Secondary branches emerge from primary branches (*a*) and exhibit retrograde growth, adhering to the main filament (*b*) and overgrowing more proximal primary and secondary branches (*c, d*), eventually fully enclosing the main filament (*e*) and forming an envelope (*f*). Anastomosis of filaments results in distinctive surface textures (*f, g*); eventually primary branches may be entirely overgrown and become indistinct (*h*). (*i–l*) Early stages of mycelial cord formation in the modern fungus *Serpula lacrymans* (Dikarya: Basidiomycota), after ref. (Butler, 1958). (*i–j*) fourth and fifth nodes behind the tip of a main hypha, showing the origin of primary (p) and secondary (s) branch hyphae. (*k*) main hypha (stippled) with a covering of narrower tendril hyphae. (*l*) tendril hyphae growing along a wide main hypha and its free primary branch (stippled). (*m*) surface texture of hyphae in *Leccinum scabrum* (Dikarya: Basidiomycota) closely resembles (*n*) surface texture of *Tortotubus*. Abbreviations as in Fig. 2. Bars, *a–l* = 10 µm; *m–n* = 2 µm.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 *Tortotubus* from the Ludfordian (Late Silurian) Burgsvik Formation, Gotland (transmitted light microscopy)

Fig. S2 *Tortotubus* from the Early Devonian of Albany, NY.

Fig. S3 *Tortotubus protuberans* from the latest Silurian Old Red Sandstone, Kerrera (transmitted light).

Fig. S4 *Tortotubus* from the latest Silurian Old Red Sandstone, Kerrera (SEM).

Fig. S5 *Tortotubus* from southeast Libya.

Further high-resolution imagery has been made available in the Dryad data repository: http://doi.org/10.5061/dryad.3r055

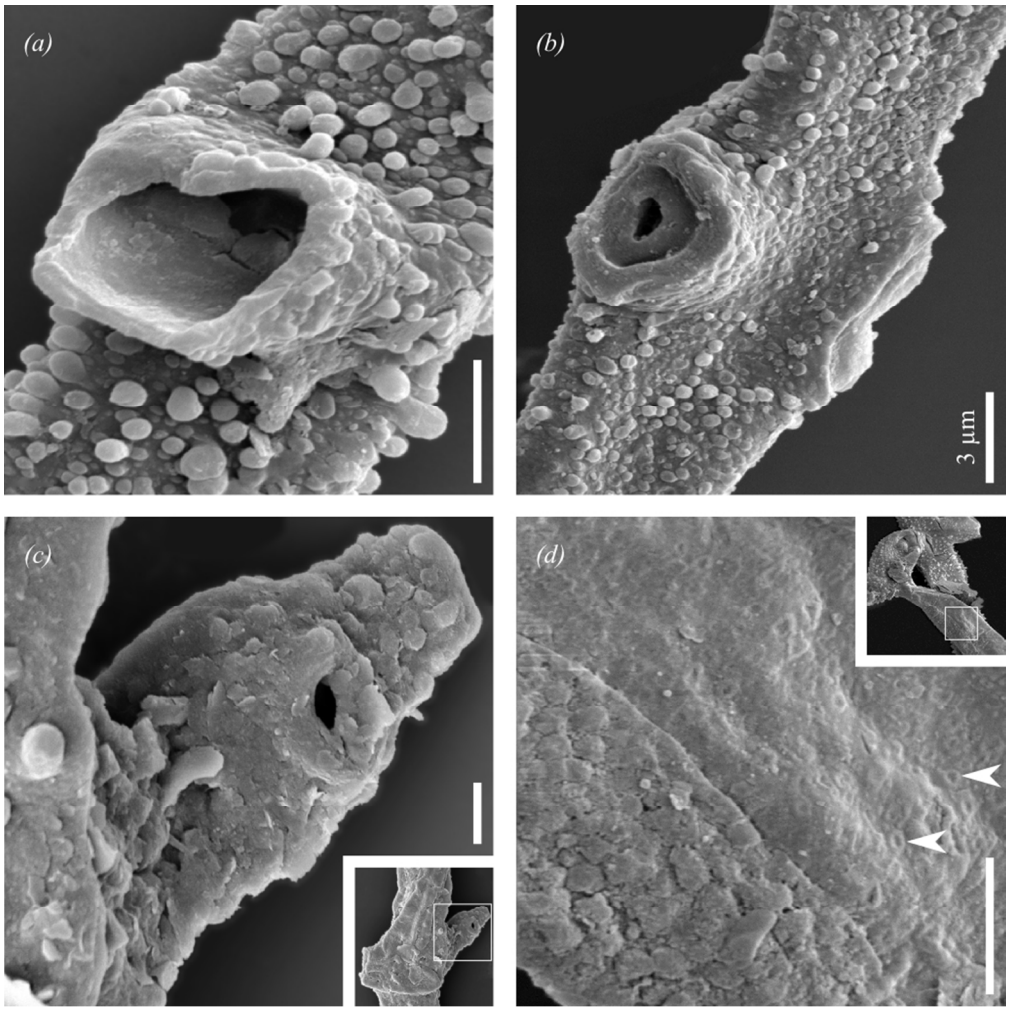

Figure 1. Tortotubus from the Burgsvik Formation, Gotland (SEM). (a) SMNH S038211-01. Globose ornament on outer cell wall; inner cell wall is smooth. (b) SMNH S142804-03. Perforate septum exposed by detachment of primary branch. (c) SMNH S038212-01. Aperture in primary branch, interpreted as attachment point of secondary branch. (d) SMNH S038211-02. Inner and outer layers of cell wall; arrowheads indicate pits with raised rims on inner wall layer. Bars, 3 µm.

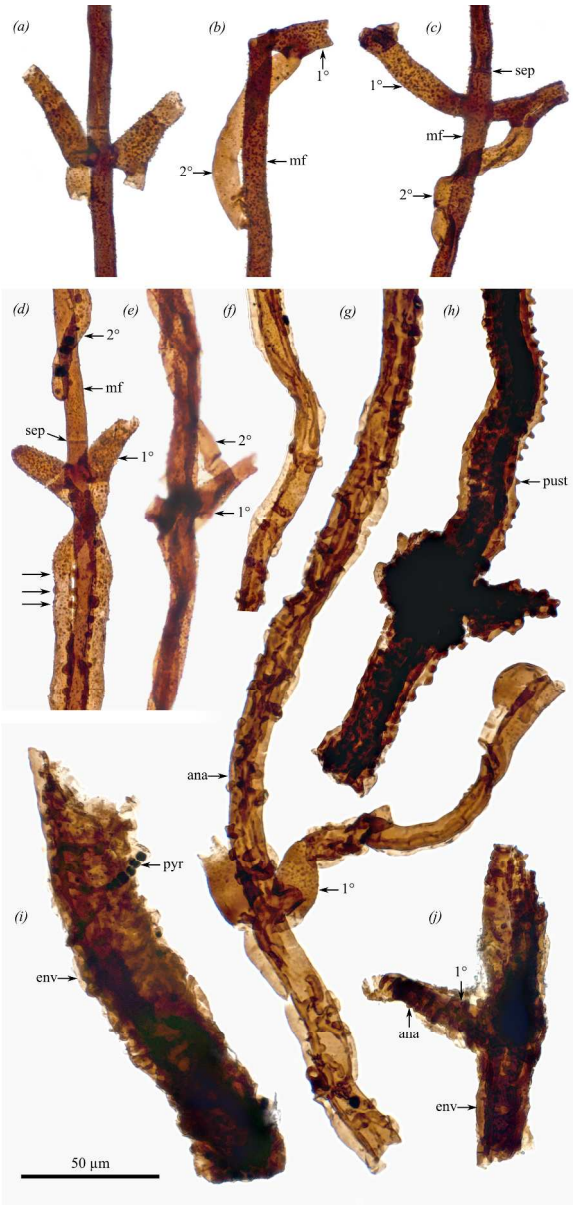

Figure 2. Development of cords in Tortotubus protuberans from the Burgsvik Formation, Gotland (transmitted light microscopy with focal stacking). Presumed growth direction to top of page. (a) SMNH S038213-01. Simple filament, with nascent secondary branches near base of primary branches. (b–c) SMNH S038214-01: (b) Secondary branch growing towards main filament; (c) secondary branch commencing retrograde growth alongside main filament. (d) SMNH S038215-01. Pustules (arrowheads) connecting secondary branches to main filament. Left-hand primary branch has intact tip. Tip of secondary branch approaching node from above. (e) SMNH S038216-01, S038217-01. Secondary branches forming envelope about main filament. (g) SMNH S038218-01. Filament with envelope of anastomosing branches. Spheroid structure at end of primary branch. (h) SMNH S038219-01. Regular pustular ornament on envelope surface. (i) SMNH S038220-01. Wide filament; a further envelope overlies anastomosing filaments and pustules. (j) SMNH S038221-01. Envelope of secondary filaments covering main filament and primary branches, ornamented by narrow anastomosing filaments. Abbreviations: 1°, primary branch; 2°, secondary branch; ana, anastomosing branches; env, envelope; mf, main filament; pust, pustule; pyr, pyrite cuboids; sep,

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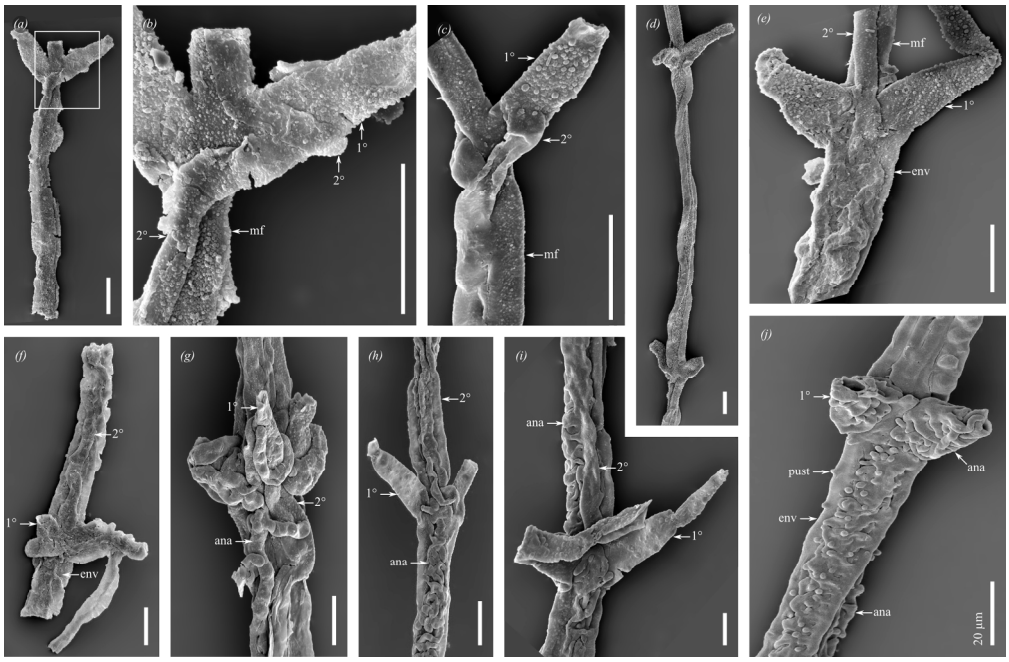
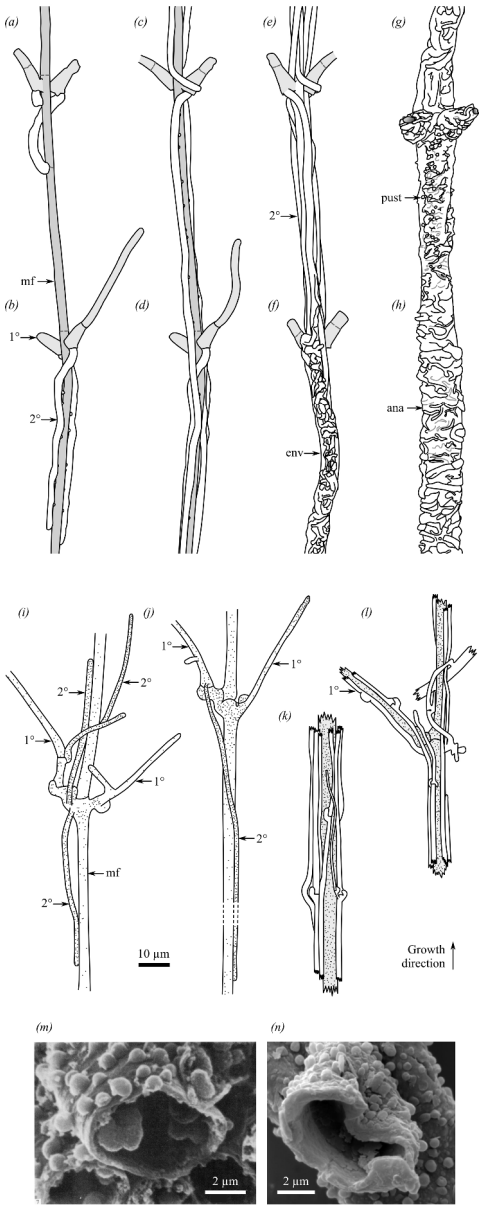


Figure 3. Cord formation in Tortotubus from the Burgsvik Formation, Gotland (SEM). (a, b) SMNH S038222-01; secondary branches propagate from basal region of primary branch and proceed down stem to next branching point. (c) SMNH S038211-03; surface of secondary branch fuses with surface of main filament. (d) SMNH S038223-01; extended filament showing extent of secondary branch growth. (e) SMNH S038224-01; secondary branches beginning to form envelope with ornamented surface; primary branches not incorporated. (f) SMNH S03821-02; envelope of secondary branches encloses primary branches. (g) SMNH S038223-02; fine anastomosing filaments on surface of envelope. (h) SMNH S038223-03; anastomosing filaments fusing to produce distinctive surface ornament. (i) SMNH S038223-04; development of surface ornament from anastomosing filaments. (j) SMNH S038225-01; surface ornament of intertwined anastomosing filaments and subregular pustules. Abbreviations as in Fig. 2. Bars, 20 µm.



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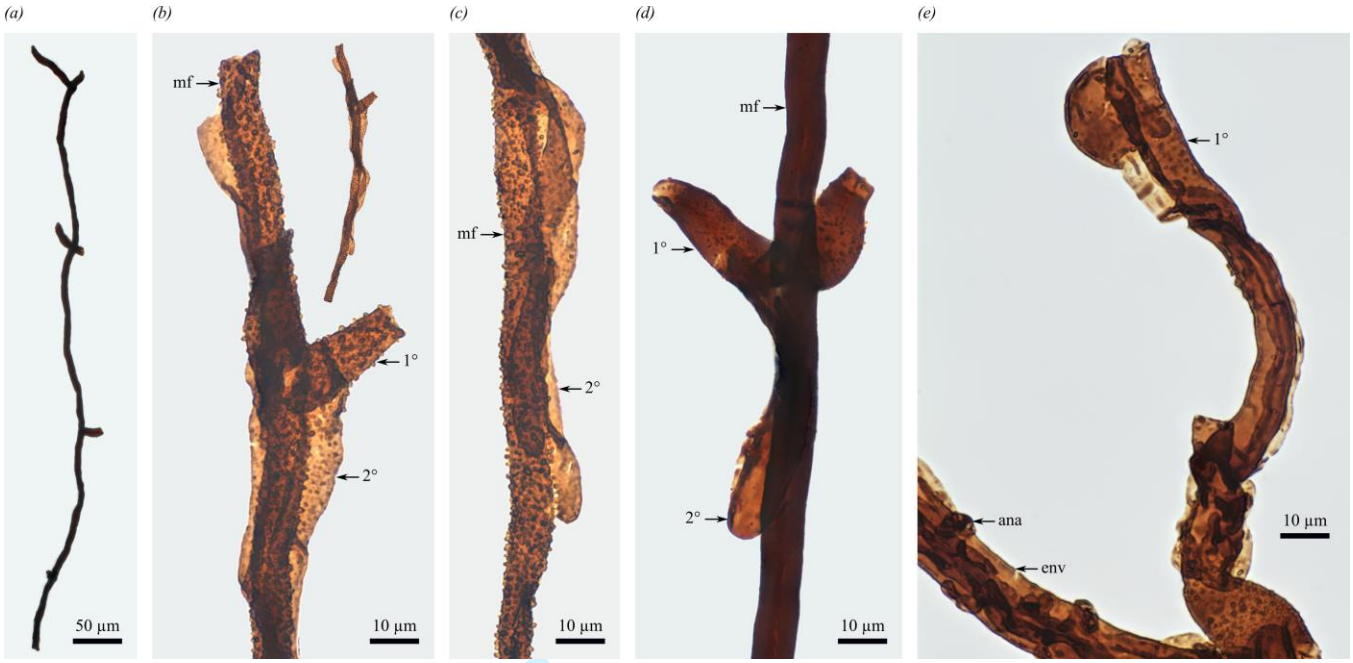
Supplementary Information

Supplementary Discussion

Wellman (1995) noted the close morphological similarity between the *Ornatifilum (=Tortotubus) lornensis* type material from Kerrera and the Gotland material reported by Sherwood-Pike and Gray (1985). Wellman noted two reasons not to treat the material as synonymous. Firstly, conspicuous perforations are not apparent in the Kerrera material. I argue that this apparent absence likely reflects the obfuscation of perforations by the high thermal alteration affecting the Kerrera material; infra-red imaging reveals perforate septa (Supp. Fig. 2p). Secondly, Wellman reports that the filament ornament is more pronounced on the Kerrera specimens than the Gotland material. This study documents substantial variation in the strength and density of ornament throughout material (Supp. Figs 1b–d, 3o–p, 4a); I did not observe any consistent difference in the ornament of material from the two localities (compare Gotland specimens in Figs 1–3, Supp. Fig. 1 with Kerrera material figured in Supp. Figs 3–4). In view of the equivalent growth trajectory expressed in material from these two localities, there can be no doubt that material from the two sites is congeneric. Material from Albany (Supp. Fig. 2) is also assigned to *Tortotubus* on the basis of its size, surface ornament, branching pattern, and formation of cords.

In view of the variability observed within individual specimens, it is difficult to distinguish genetic diversity from plasticity within a species or indeed within an individual organism. As such, I consider the present material as insufficient for a detailed treatment of species level diversity within *Tortotubus*.

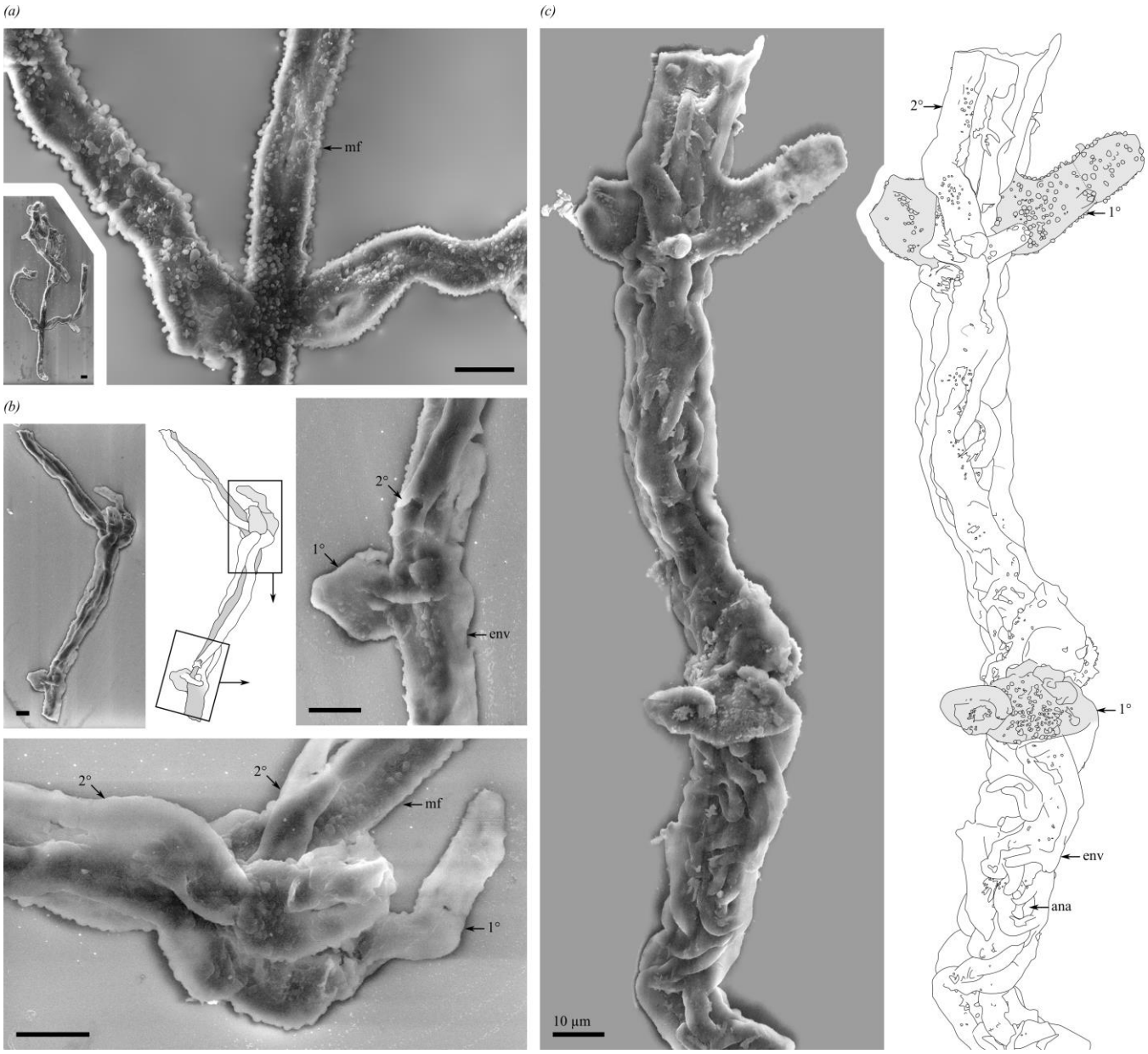


Supplementary Figure 1. *Tortotubus* from the Ludfordian (Late Silurian) Burgsvik Formation, Gotland (transmitted light microscopy with focal stacking). (*a*) SMNH XXXXX-XX, showing four consecutive branching points. (*b*–*c*) SMNH XXXXX-XX, showing variation in ornament density on secondary branches; main filament and primary branches are densely ornamented, whereas secondary branches are less ornamented and lack ornament entirely in distal regions. (*d*) SMNH XXXXX-XX; main filament and secondary branches lack ornament; primary branches are ornamented. (*e*) SMNH XXXXX-XX, enlargement of terminal spheroid illustrated in Fig. 1g. Abbreviations: 1°, primary branch; 2°, secondary branch; ana, anastomosing branches; env, envelope; mf, main filament.

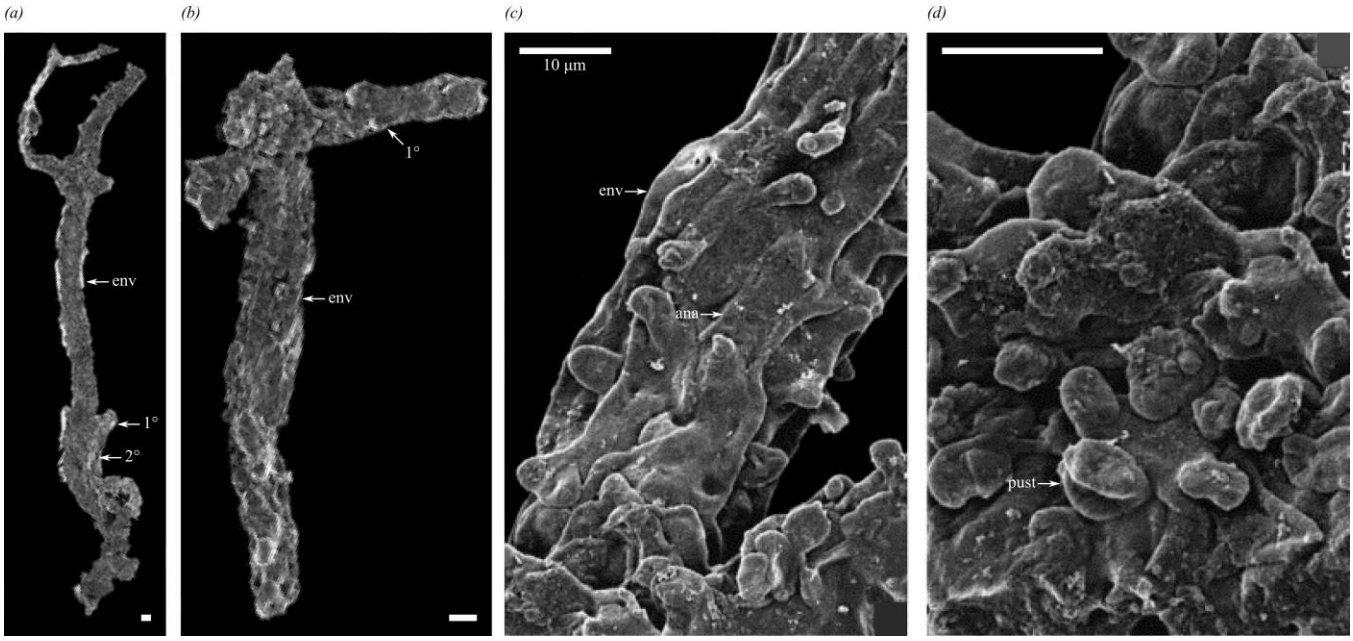
Supplementary Figure 2.
Tortotubus from the Early Devonian of Albany, NY. CAMSM X.XXXXX.XX–XX. *(a–b)* filaments showing regularly spaced primary branches. *(c–d)* strands covered by envelope of secondary branches. *(e)* Filament showing perforate septa (arrowed). Scale bars = 100 µm (a–d), 50 µm (e).

Supplementary Figure 3. *Tortotubus protuberans* from the latest Silurian Old Red Sandstone, Kerrera (transmitted light microscopy with focal stacking). Opacity corresponds to thermal maturation. Assumed growth direction to top of page. (*a–n*) CAMSM X.XXXXX.XX–XX. *(a)* secondary branches grow from primary branch and exhibit retrograde growth along main filament. *(b)* secondary filaments accumulate along main filament, leading to increase in strand width towards bottom of image. *(c)* long specimen showing regular spacing of primary branches. *(d)* primary branches protrude from nascent envelope. *(e)* secondary branch exhibiting retrograde growth; enlarged in *o*. *(f)* single filament associated with – possibly interacting with – organic material. *(g)* filament with prominent primary branches and entwining secondary branches. *(h)* wide strand possibly representing end member of *Tortotubus* development, with primary branches completely overgrown by envelope of

secondary filaments; see surface texture in *q*. *(i)* filament with prominent primary branches and broad envelope. *(j)* specimen with broad envelope. *(k)* specimen with broad envelope beginning to overgrow primary branches. *(l)* envelope bearing pustular ornament. *(m)* specimen with broad envelope. *(n)* specimen with highly developed envelope. *(o)* enlargement of *a* showing absence of ornament on secondary branch. *(p)* enlargement of *e* showing ornament on secondary branch, which exhibits retrograde growth along main filament. *(q)* SEM showing surface texture of anastomosing filaments, along with possible secondary branch, on probable strand figured in *h*. Abbreviation s: 1°, primary branch; 2°, secondary branch; ana, anastomosing branches; env, envelope; mf, main filament; pust, pustular ornament.



Supplementary Figure 4. *Tortotubus* from the latest Silurian Old Red Sandstone, Kerrera (SEM). Presumed growth direction to top of page. (*a*) CAMSM X.XXXXX.XX, showing irregular distribution of ornament. Oval region on primary branch (arrowed) interpreted as perforate septum, originally at base of now-broken secondary branch. (*b*) CAMSM X.XXXXX.XX, showing development of envelope from initially separate secondary branches. (c) CAMSM X.XXXXX.XX; secondary branches (near top) fuse into envelope adorned with anastomosing filaments (near bottom). Abbreviations: 1°, primary branch; 2°, secondary branch; ana, anastomosing filaments; env, envelope; mf, main filament. Scale bars = 10 µm.



Supplementary Figure 5. *Tortotubus* from southeast Libya (see Thusu *et al.* 2013). *(a)* filament showing two primary branching points, retrograde growth of secondary filaments, and envelope around main filament. *(b)* prominent primary branches and broad envelope. *(c–d)* surface ornament comprising anastomosing filaments and pustules. Images courtesy Giudo Meinhold. Abbreviations: 1°, primary branch; 2°, secondary branch; ana, anastomosing filaments; env, envelope; pust, pustular ornament. Scale bars = 10 µm.